MEASUREMENT OF CONTRACTION OF ISOLATED CARDIOMYOCYTES DURING ANOXIA/REOXYGENATION AND THE ANTAGONISM OF SALVIA MILTIORRHIZA

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Abstract- **The purpose of the present study was to determine the alterations of contraction parameters in isolated cardiomyocyte under anoxia and reoxygenation by using a video tracking system developed by us and examine the pharmacological application of this system to determine the effect of Salvia miltiorrhiza (SM) on anoxia and reoxygenation-induced mechanical changes. The results showed that the parameters measured were stable and consistent with those determined by other commercial device. Anoxia and reoxygenation reduced the peak velocity of cell shortening/relengthening (+/- dL/dtmax), the amplitude of contraction (dL) and the end-diastolic cell length of the isolated ventricular myocyte. Under normoxia SM alone also decreased the parameters, but under anoxia followed by reoxygenation SM attenuated the alterations of the contraction parameters. The results indicate that the video tracking system is a valuable tool for analyzing the mechanical events of cardiomyocytes and SM could protect cardiomycytes against the deterioration of contraction function induced by anoxia and reoxygenation.**

Keywords **- isolated cardiomyocyte, contraction, video tracking system, Salvia miltiorrhiza, anoxia/reoxygenation**

I. INTRODUCTION

Insight into the mechanisms of contraction and the drugs which affect the contractile status of the myocardium has been assisted enormously by the development of the enzymatically isolated cardiomyocytes as a model of cardiac function. Isolated cardiac myocytes are now widely used to study a variety of mechanical phenomena and drug screening. A variety of methods for the measurement of cell motion are currently available, which include laser diffraction techniques [1], photodiode arrays[2], video motion detectors[3]. But the limitation of using this type of system for laboratories in developing countries is the higher price.

Recently, myocardial injury induced by ischemia/anoxia and reperfusion/reoxygenation has been widespreadly concerned. In the world there are many laboratories that are searching for chemicals or natural drugs to reduce the injury of ischemia/anoxia and reperfusion/reoxygenation. Therefore there is a requirement in efficient preparation model and instruments used for screening of chemicals or drugs. Salvia miltiorrhiza (SM, or Danshen), a Chinese herbal medicine, has been used to improve blood circulation in patients with cardiovascular disorders[4]. The protective effect of SM was also observed in rat heart during ischemia and reperfusion [5]. But there is lack of experimental data from single cardiac myocyte to demonstrate the effect of SM against anoxia and reoxygenation to eliminate the influence of nervous and humoral factors as well as coronary artery.

In the present study, we used single cardiac myocyte model and a video tracking system designed for determination of effect of SM on contraction parameters in the isolated ventricular myocytes during anoxia and reoxygenation.

II. METHODOLOGY

(1) Preparation of isolated ventricular myocytes

Single ventricular myocyte from the male Sprague-Dawley rat weighing 230 ± 10g were prepared by enzymatic dissociation[6]. Immediately after decapitation, the heart was rapidly removed and rinsed in ice-cold Ca^{2+}-free Tyrode solution (in mM: NaCl 100.0, KCl 10.0, KH_2PO_4 1.2, $MgSO_4$ 5.0, Glucose 20.0, Taurine 10.0, MOPS 10.0). Then the heart was perfused via a Langendorff apparatus with a 100% oxygenated, non-recirculating Ca^{2+}-free Tyrode solution. The perfusion solution was switched to a 100% oxygenated recirculated low Ca^{2+} (50 μM) Tyrode solution containing 0.03% collagenase (type I) and 1% bovine serum albumin (BSA) for 10 min. The ventricles were cut, minced, and gently triturated with a pipette in the low Ca^{2+} Tyrode solution containing BSA at 37℃ for 10 min. The cells were filtered through 200 μm nylon mesh, resuspended in the Tyrode solution in which the Ca^{2+} concentration was gradually increased to 1.25 mM in 40 min. Only the cells with rod shaped and clear cross striations were used for experiments.

(2) Experimental setup and data processing

Fig. 1 schematically illustrates the experimental setup and the data acquisition system.

A. Experimental setup

After 1.5 h of stabilization, the isolated myocytes were moved to the 2.5 ml chamber perfused with modified Krebs-Henseleit solution (KH in mM: NaCl 118.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 1.25, Glucose 10.0, pH 7.4) with 1% BSA. A gas phase of 95% O_2/5% CO_2 was held in glass reservoir bottles and KH solution was continuously delivered to the chamber by constant flow apparatus (2 ml/min). Solution temperature in the chamber was 36± 0.3℃. The plexiglass chamber was equipped with a clear glass bottom and mounted on the stage of a microscope (IX80, Olympus). Attached to the microscope sideport was a charge-coupled device (CCD) camera for video imaging of cells in the chamber. All cell

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25OCT2001	N/A	-

Title and Subtitle Measurement of Contraction of Isolated Cardiomyocytes During Anoxia/Reoxygenation and the Antagonism of Salvia Miltiorrhiza	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Department of Physiology, Zhejiang University School of Medicine, Hangzhou 310031, China	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from the 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom., The original document contains color images.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
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measurements were obtained at × 400 magnification. None of the cells used in this study displayed spontaneous contractions in KH solution. To elicit contraction, cells were field-stimulated by using bipolar constant current pulses at 0.5 Hz, twice intensity above the threshold and 10 ms duration. The video image was analyzed on-line with a video-tracking system, in which the peak velocity of cell shortening (+dL/dtmax), the peak velocity of cell relengthening (-dL/dtmax), the amplitude of contraction (dL) and the end-diastolic cell length (L0) of the isolated ventricular myocyte were measured to evaluate the contraction of the isolated myocyte.

The O_2 scavenger sodium dithionite ($Na_2S_2O_4$) was used to establish chemical anoxia, since it provides a simple and consistent method of lowing PO_2 and maintaining low PO_2 in an open recording chamber. $Na_2S_2O_4$ was added to the glucose-free K-H solution to reach a final concentration at 1 mM and PO_2 in the solution was 0~1 mmHg, as determined with a blood/gas analyzer. Reoxygenation was achieved by returning to the normoxia K-H solution.

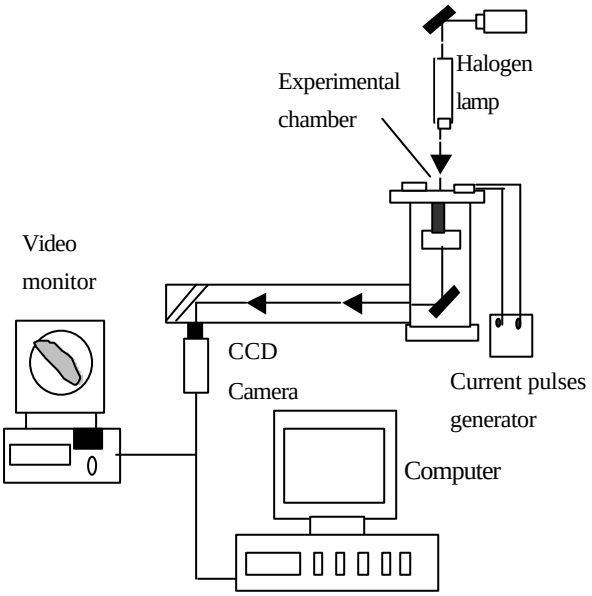


Fig. 1. Experimental setup and data recording system

B. Hardware and Software

The system comprised microscope, CCD (Pulnix TM-6710), video monitor, video digitalizer board (Meteor2-Dig/4/L) and a high-performance computer (Dell Optiplex GX110). CCD was connected to the sideport of the microscope. Video signals from CCD were transferred to a monitor and video digitalizer simultaneously. The video signals were processed with MedEase CCD (version 1.0), which was a software based on the Mil-Lite Modules (Matrox). This software was specially designed for the analysis of cell motion. More than two cells could be tracked at the same time.

C. Experimental protocol

At the end of 5 min of perfusion for stabilization, the baseline measurement was recorded. The myocyte was treated with SM accumulatively from 0.33 to 9 g/L for observation of the dose response. In another series of experiment, the myocytes were subjected to 5 min of anoxia followed by 10 min of reoxygenation to detect the response of myocyte to anoxia and reoxygenation as a control. In the drug group, myocytes were perfused with SM for 5 min, then subjected to anoxia and reoxygenation session, during which SM still existed, to detect the possible antagonistic effect of SM on anoxia and rexoygenation.

D. Data analysis and statistics

Results were expressed as means ±SE. The effects of each intervention on individual cell contractions were compared with controls by *t* tests for paired data. P value less than 0.05 was considered as significant.

III. RESULTS

A Effects of Salvia miltiorrhiza *on contraction parameters in isolated ventricular myocytes*

Under normoxic condition, contraction parameters of myocyte showed constant during perfusion with KH solution for 30 min. SM at the range of 1 to 9g/L significantly decreased dL and ±dL/dtmax in dose-dependent manner ($P<0.05$), but the contractile parameters showed no change when SM at low concentration of 0.33g/L ($P>0.05$) (Fig 2).

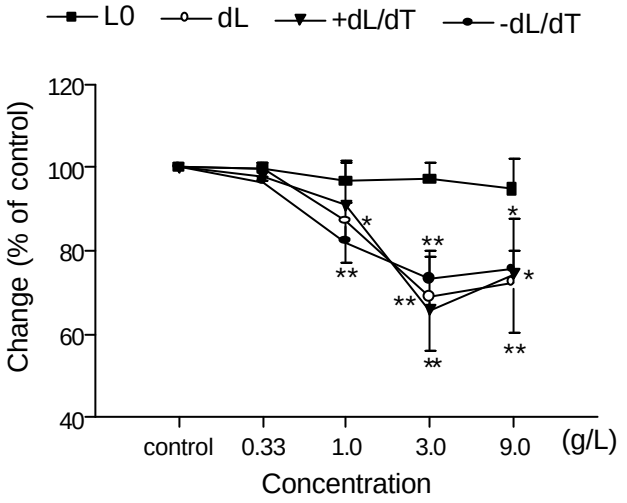


Fig. 2 Effect of SM on contraction of the isolated ventricular cardiomyocytes. Values expressed as mean±SD, n= 8~12. * $P<0.05$, ** $P<0.01$ compared with predrug value (concentration 0)

B Effects of Salvia miltiorrhiza *on the contraction parameters of the isolated ventricular myocytes during anoxia and reoxygenation*

Anoxia for 5 min decreased the dL and ±dL/dtmax significantly. Reoxygenation immediately elevated dL and ±dL/dtmax slightly, but went down gradually, lower than those during anoxia. End-diastolic cell length showed slowly decreasing but no transient recovery after starting reoxygenation. Exposure of cells to SM at 3g/L attenuated reduction of ±dL/dtmax and dL, but had no obvious effect on L0 (Figs. 3 and 4). During anoxia and reoxygenation, myocytes treated with SM beated stronger than those without SM .

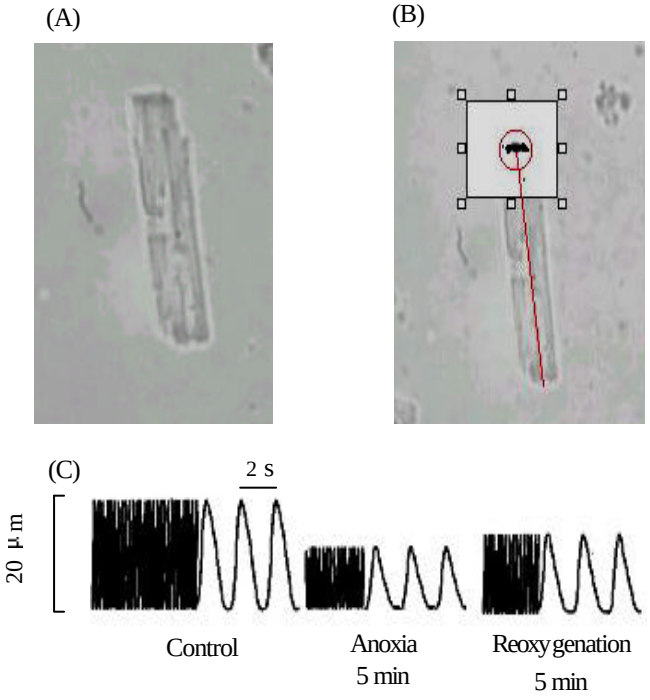
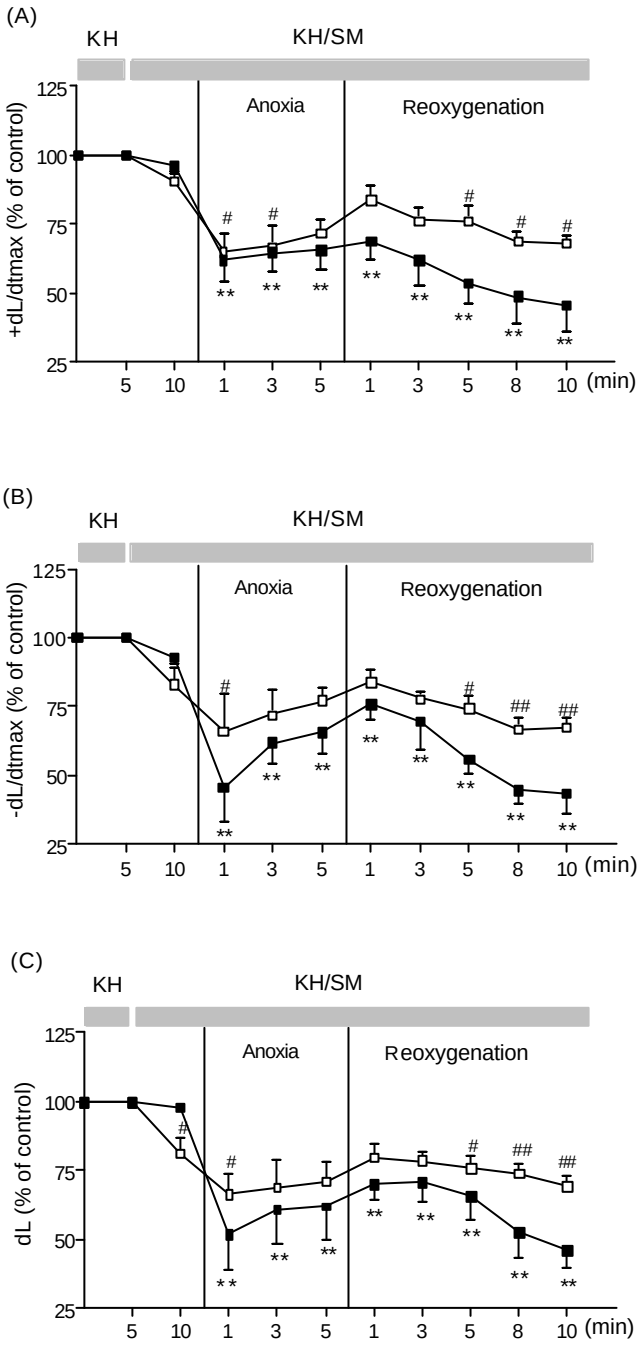


Fig. 3 Effect of SM on the contraction of the isolated ventricular cardiomyocytes during anoxia and reoxygenation. A resting cardiac myocyte (A) and a contracted myocyte (B) with video tracking frame were shown. The effect of anoxia and reoxygenation on contraction of a myocyte was displayed (C). Trace was representative of 12 experiments, all showing the same response.

IV. DISCUSSION

In the present study with a video tracking system it was shown that under normoxia condition, SM could dose-dependently inhibit inotropic status of cardiac myocyte including reduction of ±dL/dtmax and dL, enhance the tolerance of the myocytes against anoxia/reoxygenation induced alterations of the contraction parameters.

Recently, single adult isolated cardiac myocyte has become a popular experimental preparation, which has been used in numerous studies to define mechanical, electrophysiological and biochemical properties of myocytes in the absence of diffusion-limiting extracellular spaces and endogenous myocardial neurohormones[7]. At the same time, a variety of methods for the measurement of cell motion were developed,



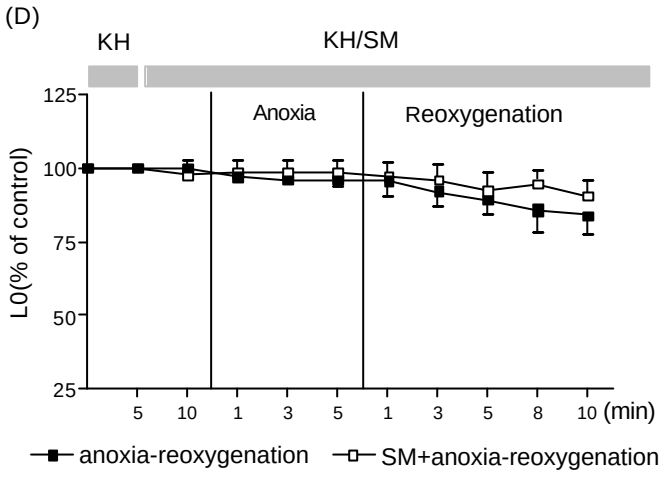


Fig. 4 Effect of SM (3g/L) on the changes of +dL/dtmax (A), -dL/dtmax (B), dL (C) and end diastolic cell length (D) induced by anoxia and reoxygenation in the isolated ventricular myocytes of rat. Values expressed as mean±SD, n= 8~12. * P<0.05, **P<0.01 compared with normoxia
# P<0.05,## P<0.01 compared with control

including laser diffraction techniques, photodiode arrays and video motion detectors. Edge detecting technique is commonly used now, but the limitation of this technique is when the target myocyte moves the system can not trace the cell automatically, which brings a big trouble for scientists. On the other hand, relative higher price for these devices is also a trouble for developing scientists. The resolution for the problem is to develop a new and high performance/cost ratio system with an automatic tracing function. The system used in the present study is a computer-based video tracking system with a powerful automatic tracing and recognizing characteristic, which was developed by our team and cheaper than those commercially. The contraction parameters of the cardiomycyte under normoxia were similar to those reported by other laboratories [8, 9].

The protective effect of SM was observed in rat heart during myocardial ischemia and reperfusion. SM treatment increased recovery of mechanical activity, improved developed pressure and increased the coronary flow rate in rat heart [5]. In the present study, we used a single cell to investigate the effect of SM in the normoxia and anoxia conditions, in which the effects of SM on the vascular endothelium could be excluded, as the cells studied were isolated adult ventricular myocytes and largely devoid of the humoral or neural influences. The " pure " effect of SM on cardiomycyte were negatively inotropic. During anoxia and reoxygenation, SM showed a beneficial effect, which attenuated the deterioration of contractile function. In a previous study, we found that SM (3g/L) could significantly protect the overload of intracellular Ca^{2+} induced by oxygen paradox in isolated ventricular myocytes of rat during anoxia-reoxygenation. So we speculate that SM may protect cardiomyocytes against the injury of anoxia/reoxygenation through decreasing the overload of intracellular Ca^{2+}.

In conclusion, the video tracking system used in the present study allows on-line quantitative determination of the contraction parameters, including shortening and relengthening, in an isolated ventricular myocyte. By using this system we found it is a valuable tool for quantitative studies of the mechanical properties of single cardiac cells in determining the effect of SM on the anoxia and reoxygenation induced alterations.

ACKNOWLEDGMENT

We thank Dr. Bruce of Department of Physiology in the University of Hong Kong for helpful advice. This work was supported by Zhejiang Provincial Natural Science Foundation of China for the Talents (RC99038).

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